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Antibacterial producing actinomycetes from Extra Andean Patagonia

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ABSTRACT

Extra-Andean Patagonia is a vast area in South America with arid soils and vegetation. Desiccation, high salinity and UV radiation of this region are considered positive factors for the isolation of bioactive strains. In the present study, this environment was screened for the first time for antibacterial producing actinomycetes. The isolated strains included members of the genera *Streptomyces*, *Micromonospora*, *Microbacterium*, *Nocardia*, *Dermacoccus*, and *Dietzia*. Among them, four antibacterial producers belonging to the genus *Streptomyces* were detected and one isolate belonging to the genus *Micromonospora* harbored the 3-amino 5-hydroxybenzoic acid synthase gene involved in the production of ansamycin-like compounds. The most promising isolate, *Streptomyces* sp. SUE01, produced pigmented antibiotics of the granaticin class. Since this type of metabolites has been previously isolated from strains growing in desert environments, a relationship between prevailing environmental factors in arid ecosystems and secondary microbial metabolism could be suggested.

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For many years, actinomycetes provided the most successful molecules for chemotherapy. In the past decades, intensive screening programs have been performed in such a way that the rediscovery of previously known molecules increased. The failure in finding novel compounds from actinomycetes was attributed to a depletion of isolating, extracting, and testing methods. Since there is a constant need of finding antibiotics and chemotherapeutic agents with better pharmacological profiles, new strategies for finding novel chemical bioactive structures have been developed in the past decades. Those include genomic screening and bioprospecting in unusual environments or underexplored bacterial taxonomic groups. It is believed that ecological and environmental conditions influence the metabolic capability of bacteria towards the production of bioactive compounds (Mohammadipanah and Wink, 2016). In that context, bacteria inhabiting underexplored ecosystems would be a source of new bioactive metabolites. Accordingly, bioprospecting in marine environments for bioactive strains successfully afforded new chemical entities over the past years (Mohammadipanah and Wink, 2016). More recently, there has been an increasing interest in exploring arid environments where bacteria are exposed to extreme conditions such as desiccation, high salinity, and UV radiation (Mohammadipanah and Wink, 2016; Bull and Asenjo, 2013). Bioprospecting in such environments led to the isolation of novel bioactive metabolites such as chaxamycins (Rateb et al., 2011) and abenquines (Schulz et al., 2011). These metabolites are related to the ansamycin family of antibiotics, structurally defined as metabolites with an aromatic ring bridged by an aliphatic chain. As an example, Rifamycin S is one of the most famous members of this class of antibiotics. The biosynthesis of such compounds involves the 3-amino 5-hydroxybenzoic acid (AHBA) synthase which has been used as a target for the genomic screening of ansamycin producers isolated from hyper-arid environments (Rateb et al., 2011).

Patagonia is located at the southern region of South America and covers an area from latitude 37 S to 56 S. The band extending to the east of the Andes is known as "Extra-Andean Patagonia," which covers an area of approximately 550,000 km^2. The Extra-Andean region has an arid to semi-arid climate, temperate to cold temperatures (mean annual temperature 12 °C), and steppe vegetation adapted to arid conditions (Paruelo et al., 1998). The microbial diversity inhabiting this region represents a valuable source of biotechnological products (Vela Gurovic, 2016). However, the bioactive potential of actinomycetes from this region towards the production of antibacterial agents has not been explored to date. In this study, we aim to assess the potential of actinomycetes isolated from underexplored arid environments in Extra-Andean Patagonia, Argentina, towards the production of novel bioactive metabolites.

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Additionally, a genomic screening has been performed to detect the presence of AHBA biosynthetic genes involved in the production of ansamycins.

The sampled region is located in northeastern Patagonia, where the annual mean precipitation is between 100 and 200 mm with winds between 14 and 20 km/h. The percentage of radiation is about 50% of sunshine hours (Paruelo et al., 1998). Since this area of Patagonia is sometimes under the influence of the Antarctic ozone "hole", it occasionally receives enhanced levels of ultraviolet B radiation. Typical vegetation includes a grassy-shrubby steppe of medium height and density, distributed in a pattern of plant patches alternating with bare soil areas (Mazzonia and Vazquez, 2009). Soil samples (0–10 cm depth) were collected from different points in the Chubut Province (Argentina), between 2012 and 2013. The sampling covered different geographic points around the city of Puerto Madryn, the coast nearby, and the lower Chubut River valley. The coordinates (latitude/longitude) of the sampling points were: site A -43° 20′ 54″/-65° 40′ 39″; site B -42° 39′ 44″/-64° 59′ 24″; site C -42° 80′ 10″/-64° 93′ 70″. In site C we collected soil under plant-covered patches and at intercanopy areas of bare soil along with standing leafs of typical species of the region (Table 1). All samples were dried at 80 °C for 1 h to promote the isolation of spore-forming actinomycetes. Thereafter, aliquots of approximately 1 g were suspended in 20 ml of sterile saline solution and they were shaken to detach bacteria. Serial dilutions were plated in humic acid-vitamin agar supplemented with nystatin 50 μg/mL and nalidixic acid 20 μg/mL, and incubated at 28 °C for a month (Hayakawa and Nonomura, 1987). A total of 58 isolates were obtained from soil and vegetation samples. The isolates were further cultivated on yeast extract/malt extract agar plates (ISP-2) for 3 weeks at 28 °C. Colonies were collected from the agar plates and inoculated in liquid media for 4 days at 28 °C and 220 rpm. The supernatants from cultures in ISP-2 and production medium containing 2% D-sucrose, 0.2% casitone, 0.5%, cane molasses, 0.01% $FeSO_4$. $7H_2O$, 0.02% $MgSO_4$. $7H_2O$, 0.05% NaI and 0.5% $CaCO_3$ (Vela Gurovic et al., 2013) were tested for antibacterial activity against *Bacillus subtilis* subsp. *subtilis* str. 168 and *E. coli* ATCC 25922 (Table 1). Isolates were also subjected to genomic screening to detect the presence of AHBA biosynthetic genes (Huitu et al., 2009). Isolates producing antibacterial activity and randomly selected negative isolates from each sampling site were subjected to 16S rRNA analyses for taxonomic affiliation (Table 1). Almost 500 bp of the 16S rRNA gene of the isolates were amplified using the primers 27f and 518r (Olivera et al., 2007). Sequencing of the amplified fragments was performed by the commercial services of CENPAT Molecular Biology Laboratory (Argentina). The partial 16S rRNA gene sequences were compared with public sequences in the EzTaxon extended databases (Kim et al., 2012). Sequences were deposited into the GenBank database under the accession numbers KX170839–49 (Table 1). The accession number of the AHBA sequence of the CEBF isolate was KX189122. Furthermore, the isolate which showed the highest antibacterial activity against Gram positive and negative bacteria, SUE01, was inoculated in producing media for 4 days at 30 °C and 250 rpm. The supernatant was concentrated under reduced pressure and subsequently extracted with methanol and acetone to isolate the active compounds by a bioguided assay based on the agar diffusion test against *B. subtilis*. Active compounds were purified by HPLC (Waters 600 Controller Chromatograph equipped with a diode-array detector, ZORBAX C18 Prep 10 μm column, flow 1 ml/min, eluting in a gradient from 65% to 100% acetonitrile in water).

The AHBA gene was detected only in isolate CEBF (KX189122), which belonged to the genus *Micromonospora*. After running BLAST analyses, this sequence showed 92% of similarity with the AHBA gene Micau_2448 of *Micromonospora aurantiaca* ATCC 27029 (T). Contrary as expected, CEBF did not show antibacterial activity (Table 1). This lack of activity could be explained by the low production of the metabolite, a silent state of the biosynthetic gene cluster, or the lack of additional genes needed for the biosynthesis of the antibiotic (Huitu et al., 2009). The fact that all the isolates

Table 1
Origin, taxonomy and antibacterial activity.

Selected strain (accession number)	Origin	Sample site (N° of isolates)	Genus affiliation	Type strains with highest sequence similarity[a]	Bioassay[b]	
					G+[c]	G-[d]
CEBF (KX170839)	**soil**	A (13)	*Micromonospora*	*Micromonospora echinofusca* DSM 43913 (T) (99.51)	-	-
D1A (KX170840)	**soil**	B (2)	*Streptomyces*	*Streptomyces bellus* ISP 5185 (T) (100.00)	++	++
SO2 (KX170841)	**soil**	C (28)	*Nocardia*	*Nocardia coubleae* NBRC 108252 (T) (97.55)	-	-
SO4 (KX170842)	**soil**		*Microbacterium*	*Microbacterium foliorum* DSM 12966 (T) (98.80)	-	-
SUE01 (KX170843)	**soil**		*Streptomyces*	*Streptomyces lateritius* LMG 19372 (T) (99.15)	+++	++
SUE08 (KX170844)	**soil**		*Streptomyces*	*Streptomyces nojiriensis* LMG 20094 (T) (100.00)	+	-
3501 (KX170845)	**soil**		*Streptomyces*	*Streptomyces lienomycini* LMG 20091 (T) (99.75)	-	-
	plant source					
AX1 (KX170846)	*Atriplex lampa*	C (2)	*Dietzia*	*Dietzia shimae* YIM 65001 (T) (100.00)	-	-
CH3 (KX170847)	*Chuquiraga avellanedae* Lorentz	C (2)	*Dermacoccus*	*Dermacoccus nishinomiyaensis* DSM 20448 (T) (99.01)	-	-
PO7 (KX170848)	*Prosopis alpataco* Phil.	C (11)	*Streptomyces*	*Streptomyces tendae* ATCC 19812 (T) (100.00)	-	-
PR6 (KX170849)			*Streptomyces*	*Streptomyces spinoverrucosus* NBRC 14228 (T) (99.75)	+	-

[a] Similarity is given in brackets.
[b] Activity was rated by the diameter of the halos (>10 mm +; >15 mm ++; >20 mm +++).
[c] *B. subtilis* subsp. *subtilis* str. 168.
[d] *E. coli* ATCC 25922.

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